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Non-specific effects of vaccines: plausible and potentially important, but implications uncertain

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## Abstract

*Non-specific effects (NSE) or heterologous effects of vaccines are proposed to explain observations in some studies that certain vaccines have an impact beyond the direct protection against infection with the specific pathogen for which the vaccines were designed. The importance and implications of such effects remain controversial. There are several known immunological mechanisms which could lead to NSE, since it is widely recognised that the generation of specific immunity is initiated by non-specific innate immune mechanisms that may also have wider effects on adaptive immune function. However, there are no published studies that demonstrate a mechanistic link between such immunological phenomena and clinically relevant NSE in humans. Whilst it is highly plausible that some vaccines do have NSE, their magnitude and duration, and thus importance, remain uncertain. Although the WHO recently concluded that current evidence does not justify changes to immunisation policy, further studies of sufficient size and quality are needed to assess the importance of NSE and the effects of different sequences of vaccinations on all-cause mortality. This could provide insights into vaccine immunobiology with important implications for infant health and survival.*

## Introduction

In 1974, the World Health Organization (WHO) recommended six vaccines (diphtheria, pertussis, tetanus, measles, poliomyelitis and tuberculosis) for immunisation of infants, with a target set in 1977 that this Expanded Programme on Immunization (EPI) should reach all children by 1990 ([www.who.int/immunization/programmes\\_systems/supply\\_chain/benefits\\_of\\_immunization/en/](http://www.who.int/immunization/programmes_systems/supply_chain/benefits_of_immunization/en/)). Despite considerable progress, that goal has not yet been achieved, but today more than 85% of children receive these vaccines and many others, including hepatitis B, *Haemophilus influenzae* type b (Hib), rotavirus (RV) and pneumococcal conjugate vaccines (PCV). The impact of immunisation against these diseases is astonishing with estimates that 2-3 million deaths are prevented every year as a result of national vaccine programmes<sup>1</sup>. Vaccination works because the immune system engages defences against the antigenic components in the vaccine to produce a high quality, rapid and

specific immune response against the relevant pathogen. In addition to this specific effect, some researchers have argued that there are additional, non-specific effects of vaccines, beyond the direct protection against the diseases for which the vaccines were developed.

These “non-specific effects” (NSE) of vaccines have also been termed “heterologous effects” or “off-target effects”. The main outcome, which has been considered in epidemiological studies of the NSE of vaccines, is all-cause mortality with some authors concluding that, until a different vaccine is given, there are substantial additional reductions in all-cause mortality with live vaccines (such as oral polio vaccine (OPV), Bacillus Calmette–Guérin (BCG) and measles vaccines) and, conversely, increased all-cause mortality (in high mortality settings) with non-live vaccines (such as inactivated polio vaccine (IPV) and diphtheria tetanus & whole cell pertussis (DTP) combined vaccines). There is some evidence that these NSE on all-cause mortality are generally stronger in females than males <sup>2</sup>. Furthermore, it is argued that, if the most recent vaccine received is a non-live vaccine, the non-specific benefits obtained from a prior live vaccine are negated <sup>3</sup>. The topic has been controversial, as much of the evidence is from studies with high risk of bias <sup>4</sup>, has been addressed in few randomised controlled trials (RCTs) and arises from a limited range of settings (dominated by studies from Guinea Bissau). Furthermore, polarisation of views on the subject has complicated the assessment and unbiased interpretation of the existing data.

Some findings relating to the NSE of vaccines, notably the association between some live vaccines and a pro-inflammatory protective state, are immunologically compelling. In addition, a large number of animal studies support a beneficial effect of BCG in enhancing the immune response to unrelated pathogens <sup>5</sup>. It is plausible that a vaccine given shortly after birth, such as BCG, may influence the developmental trajectory of the immune system during a critical window of maturation. However, if NSE do occur as a result of vaccination later in infancy and have an important impact on mortality, it seems likely that the many other unpredictable bacterial and viral infections of early childhood, especially in settings with a high infectious burden, would also have non-specific effects on morbidity and mortality. With this in mind, it might be argued that NSE would be overwhelmed by greater exposure to these other pathogens than to the vaccines themselves. Furthermore, in observational studies, other differences between the populations who receive the vaccine(s) of interest and those who do not (such as healthcare-seeking behaviour) may increase the risk of bias.

In 2016, a systematic review by Higgins *et al.*, commissioned by the World Health Organization, was published <sup>6</sup> which attempted to evaluate whether there was evidence to support a change in the EPI programme as a result of NSE of vaccines with reference to BCG, DTP and measles vaccines. The findings of this review are briefly reviewed below. WHO concluded that there was insufficient evidence to support changes to current global immunisation policy<sup>7</sup>.

## **BCG**

In Higgins *et al.*'s systematic review, vaccination with BCG was shown to be associated with a reduction in all-cause mortality of around 50%, substantially greater than anticipated from prevention of tuberculosis alone. This was the case both in two RCTs in low birth weight infants considered at low risk of bias (relative risk (RR) 0.52, 95% confidence intervals (CI) 0.33 to 0.82), in two RCTs conducted in Native American children in the 1930s and 40s, and in nine observational studies considered at high risk of bias (RR 0.47, 95% CI 0.32 to 0.69) <sup>6</sup>. There was no difference in the effect between boys and girls, and the benefit seemed to decrease with age. Other recent studies have suggested a beneficial effect of BCG vaccination on reducing (non-tuberculosis) respiratory infections. A population-based analysis from 33 countries over 25 years showed that BCG-vaccinated children had a 17-37% lower risk of acute lower respiratory tract infections <sup>8</sup>. Similarly, a large

retrospective observational study from Spain found that there was a 40% lower rate of hospitalisation for respiratory infections and 36% decrease in sepsis admissions among BCG-vaccinated children, but the vaccinated and control groups were from different regions and it was not possible to control for confounding<sup>9</sup>.

Whilst it is plausible that BCG prevents a substantial proportion of non-tuberculous infant deaths in certain settings, it is not clear how this observation is affected by the particular strain of BCG used<sup>10</sup> (several different vaccine strains are used around the world), timing of immunisation (BCG is given from birth to several months of age), or to survival of term infants born in different geographic regions. Follow-up duration in studies was typically short and it is not possible to assess whether the described effects persist beyond the first year of life. If beneficial NSE are induced by BCG, vaccination may be especially important in the neonatal period in high mortality settings where many deaths occur in the first month of life.

It seems less likely that there are profound NSE associated with BCG vaccination in low mortality settings: in a recent RCT in Denmark, no reductions in hospitalisation for somatic acquired disease or parent-reported infection was documented in the first year of life amongst those who were randomised to receive BCG when compared with controls<sup>12</sup>. However, in a pre-specified subgroup analysis, neonatal BCG vaccination may have reduced infections in the first three months of life in children whose mothers were BCG-vaccinated<sup>13</sup>. There therefore remains uncertainty about the importance and applicability of the NSE of BCG in different populations today. Further research, especially on impact in the neonatal period in low-income settings, where mortality from sepsis is high, might lead to the strengthening of recommendations for neonatal doses of BCG vaccine in some countries. The significance of NSE of BCG vaccination are also of potential importance for new tuberculosis vaccines designed to replace BCG and for decisions in relation to the discontinuation of routine BCG vaccination in countries where the decreasing incidence of tuberculosis no longer justifies its use for its specific effects.

## **Measles**

Four RCTs which considered all-cause mortality following measles vaccine and 18 observational studies were included in the systematic review<sup>6</sup>. The observational studies indicated a substantial reduction in all-cause mortality following measles vaccine (RR 0.51, 95% CI 0.42 to 0.63) but all were considered at high risk of bias. The evidence from the RCTs was not statistically significant (RR 0.74, 95% CI 0.51 to 1.07). Observed effects were greater in girls than in boys. Although the data on NSE of measles vaccine remain uncertain from the available studies, if measles vaccine does have beneficial NSE, vaccination may be an important contributor to mortality reduction in young children in low-income settings. Mina *et al*<sup>14</sup> have suggested that, following recovery from measles, there is temporary loss of immune memory and a consequent increased risk of mortality. They modelled epidemiological and immunological data and found that, when measles was common, almost 50% of infection-related deaths in childhood could be attributed to a prolonged post-measles disease immunosuppressive effect. Since measles vaccine prevents measles, and therefore the post-measles infectious mortality, vaccination would have benefits beyond prevention of measles itself. Interestingly, this study indicates an overall effect size similar to that seen in the systematic review of the evidence for NSE. WHO currently advises measles vaccination after 9 months of age and the data currently available did not provide evidence for a change in global policy. If NSE are as important as some of the data summarised above suggest, earlier immunisation might further reduce mortality before 9 months of age. However, since maternal antibodies influence the immune response in younger infants and since the size and duration of the measles antibody response induced by the vaccine increases with age and immunological maturity independently of maternal

antibody effects, with earlier immunisation measles protection could be compromised leading to an overall increase in measles and post-measles mortality.

### **Diphtheria, tetanus and pertussis**

Diphtheria, tetanus and pertussis are important contributors to child mortality in the absence of vaccination, with one recent analysis from the Netherlands indicating that 5.3% of all childhood deaths were attributable to these diseases in that country in the pre-vaccine era<sup>15</sup>. The systematic review by Higgins *et al* found no RCTs that evaluated the effect of DTP vaccination on all-cause mortality<sup>6</sup>. Ten observational studies were analysed, all of which were considered to have high risk of bias, finding an apparent increased mortality rate with an average RR of 1.38 but with 95% CIs that crossed 1.0 (0.92 to 2.08). Subsequently, Higgins *et al* acknowledged concerns with one of the included studies; with this study excluded, and the use of a random effects meta-analysis, their estimate of the RR of mortality for DTP is 1.53 (95% CI 1.02 to 2.30)<sup>6</sup>. Most of the studies included had an observation period of less than 8 months. Of the ten, two (including the study with major concerns about its bias) found an overall protective effect, one showed no apparent effect of the vaccine on all-cause mortality, and most did not find evidence of a difference between males and females. Mina *et al*<sup>14</sup>, in the analysis mentioned above, also analysed whether pertussis disease had any effect on non-pertussis mortality and were not able to show any relationship. The implications of these findings are limited by a dearth of high quality studies. Furthermore, an increasing proportion of children in low-income settings are now immunised with the pentavalent vaccines (DTP combined with hepatitis B and Hib) and many with schedules that include other vaccines such as PCV and RV, all of which could modify any NSE. WHO concluded that the available evidence does not support any global immunisation policy changes. This conclusion has provoked heated debate. Concern has been raised about the selection of studies included in the review, and the suggestion that bias may have contributed to the findings in observational studies has been disputed<sup>16</sup>. Protagonists of deleterious NSE of DTP argue that biases in observational studies ought not to operate in opposite directions for different vaccines and that frailty bias (where clinics do not vaccinate a child who is sick) would tend to reduce any apparent adverse effects of DTP. Conversely, antagonists point out that reaching conclusions from non-randomised studies is unsafe and that existing data are not sufficiently robust to drive changes in policy. The polarised views about the NSE of DTP can only be resolved by randomised trials done in relevant low-income settings. Design of studies of the NSE of vaccines is currently being considered by WHO.

### **Polio**

A recent RCT evaluated the effect of a birth dose of OPV on all-cause mortality in Guinea Bissau and found no statistically significant effect of the vaccine on mortality (hazard ratio 0.83; 95% CI 0.61-1.13)<sup>17</sup>. Various sub-analyses found some evidence of significant NSE, particularly if the vaccine was given early, but this study does not provide conclusive evidence for an effect of OPV in reducing all-cause mortality. Since OPV can cause vaccine-associated paralytic poliomyelitis (VAPP) and live polio viruses are currently being eradicated globally, live OPVs will be progressively replaced by IPV in the years ahead as part of the global polio eradication plan.

### **Rabies**

A hypothesis has recently been proposed to explain higher numbers of cases of meningitis of different aetiologies and of cerebral malaria among children receiving the RTS,S malaria vaccine than in controls who received (non-live) rabies vaccine in a recent large RCT<sup>18</sup>. Specifically, it has been suggested that, rather than the result of chance, or of a rare adverse effect of the RTS,S vaccine, these findings might be attributable to protective NSE of rabies vaccine<sup>18 19</sup>. A small number of observations in animal studies support this theory, which might be explained by superantigenic

properties of the rabies nucleoprotein. Further experimental studies are needed to explore the plausibility of this idea, which, if proven, would be an example of beneficial NSE of non-live vaccine antigens.

### **Sex**

An important aspect of NSE has been the apparent differences between the size of effects in male and female infants. Differences between sexes in the immune responses to vaccines are well-recognised with females generally, although not in all studies or for all antigens, making stronger specific antibody responses than males<sup>20-23</sup>. Hormonal differences may explain this in part, since sex hormone receptors are found on cells of the immune system<sup>22</sup>. A study of yellow fever vaccine showed greater gamma-interferon pathway gene upregulation in women than men in the first 10 days after vaccination<sup>24</sup>. It is therefore plausible that there are clinically relevant differences between male and female infants in NSE, although any impact of these on survival, and thus implications for immunisation policy remain uncertain.

### **An immunological basis for non-specific effects of vaccines**

Since Edward Jenner's experiments with cowpox that led to the birth of vaccination, it has become widely understood that administration of an attenuated or inactivated bacterium, virus or components of a pathogen can lead to induction of specific immunity to that organism that will protect the individual on subsequent exposure and may also reduce spread of infection and thus provide herd protection. Since Jenner's time, considerable advances have been made in the understanding of the generation of antigen-specific immunity and, more recently, the initial engagement of the innate immune system (following recognition of "danger signals" such as bacterial surface products or DNA), which promote the induction of adaptive T and B cell immunity. While the adaptive immune response is highly specific to the immunising agent or closely related antigenic structures, the early or innate immune response is more promiscuous. For example, interferon-alpha produced in response to a viral infection makes cells more resistant to subsequent infection by another viral agent. It is well-recognised that the nature of the initial innate response can determine the character and outcome of an immune response, a fact that has been harnessed in the development of vaccine adjuvants to enhance and direct protective responses with new vaccines. In addition, vaccine components with superantigenic properties, which can induce polyclonal T cell activation and T cell bystander effects provide additional known mechanisms which could theoretically result in NSE. The existence of such non-specific immunological effects (NSIE) are central to contemporary understanding in immunology. Furthermore, both infectious diseases and vaccines induce epigenetic changes which may affect the subsequent behaviour of cells involved in the immune response<sup>25</sup>.

The most compelling evidence for NSIE with importance for protection against infectious diseases comes from a number of studies in animals over the last half century which have shown that there is T cell dependent resistance to unrelated pathogens which can be induced in mice<sup>5 25</sup>. For example, mice that were latently infected with gamma-herpes virus developed prolonged production of gamma-interferon, macrophage activation and were resistant to infection with listeria or yersinia<sup>26</sup>. Similarly, mice infected with *Brucella abortus* were resistant to listeria infection, with resistance correlating with macrophage phagocytic activity, although, in these elegant experiments, it is noteworthy that resistance was short-lived (demonstrated at day 18 after brucella infection but not present at day 4 or day 35)<sup>27</sup>. BCG vaccination protects mice against murine malaria<sup>28</sup> and also induces protection against both candida<sup>29</sup> and schistosoma<sup>30</sup> infection which is independent of T and B cell activity. While the mechanisms of resistance are not clear, there is evidence that epigenetic modifications occur as a result of exposure to some bacterial and fungal products.

‘Trained immunity’, the concept that epigenetic reprogramming can enhance the innate immune response to reinfection, has been proposed as a mechanism that might be involved in NSE<sup>31</sup>. One study found that increased production of pro-inflammatory cytokines by human NK cells was still present 3 months after BCG vaccination<sup>32</sup>.

Such studies in humans, linking disease endpoints to NSIE observations, are lacking and it is not currently possible to determine whether induction of particular cell types, cytokines or other mediators will improve clinical outcomes. In a recent review of immunological data that were available from studies of infants following BCG, DTP or measles vaccination, formal meta-analysis was not possible due to the heterogeneity in study design<sup>33</sup>.

In this systematic analysis of NSIE, undertaken as part of the WHO’s review of NSE, increased production of gamma-interferon after BCG vaccine was the most consistent finding in various *in vitro* experimental conditions, although some studies showed no effect<sup>33</sup>. Overall, the findings, though highly variable, were in line with observations in animal studies that BCG vaccine induces a more pro-inflammatory state. It is unclear whether this translates to increased resistance to future infection and it is not clear how long such effects might last or whether they can be consistently induced in routine vaccine programmes.

Some consistent immunological effects have been identified following measles vaccination with a few studies showing increases in various cytokines while others showed no effect or even diminished responses. Interferon-gamma production *in vitro* was increased in some studies but unaffected or reduced in others. *In vitro* lymphoproliferation was increased after measles vaccination in response to candida and tetanus antigens and in some unstimulated cultures. The implications of these findings are unknown.

There have been few immunological studies addressing DTP or its components and there were no consistent findings. One study found an increase in production of IL-5 and another found an increase in gamma-interferon production following stimulation of cell cultures. Several studies reported increases in unrelated IgG antibody levels (eg. serotype 14 pneumococcal antibody). These findings are difficult to interpret.

Studies of NSIE in humans indicate that there may be some measurable changes in cytokine production, lymphocyte activation or cell numbers following vaccination but, for the present, it is not possible to determine from the literature whether such effects can be consistently induced, when they can be expected to occur, for how long they persist or, indeed, whether they have any clinical consequences.

### **The live vs non-live vaccine hypothesis**

Since beneficial NSE have been attributed to BCG and measles vaccines, it has been proposed that live vaccines may have particular properties, beyond target disease prevention, which reduce infant mortality, whereas non-live vaccines may do the opposite<sup>2316</sup>. A number of live organisms and live-attenuated vaccines induce production of pro-inflammatory cytokines (the most studied is gamma-interferon as discussed above), which could, in turn, direct enhanced innate immune protection as described in various animal studies. However, this is likely to be an over simplification as there is wide variation in induction of pro-inflammatory responses by different live organisms. Indeed, non-live organisms, vaccines and adjuvants can also induce inflammatory cytokine responses. Perhaps the most interesting concept here is the “setting of the thermostat” idea, that a birth dose of BCG may better set the immune system, perhaps through epigenetic changes, to produce protective

responses when challenged by infection in the subsequent months<sup>34</sup>. This idea requires investigation in clinical trials.

## Conclusions

Current immunobiological perspectives and the widespread use of vaccine adjuvants to stimulate the innate immune system and enhance vaccine immunogenicity are evidence that there can be NSE of vaccines, even though our understanding of the importance, magnitude and duration of impact remains inadequate. In particular, interventions designed to augment non-specific resistance to life-threatening infections during the first few vulnerable months of life, particularly in high mortality settings, merit further investigation. Improved understanding of these effects is also fundamental for the future development of improved, safe and less reactogenic vaccines and so remains an important area for research. It is important to note here that the mechanisms underlying the specific effects of many vaccines remain poorly understood, including, for example, BCG vaccine's protective effect against tuberculosis.

The potential for adding benefit to current vaccine programmes by reducing mortality beyond the targeted diseases is clearly a worthy goal. In this context it is worth noting that the importance of 'indirect effects' of vaccines – where high vaccine coverage decreases transmission with reduced incidence of target diseases in unimmunised as well as immunised individuals – is universally acknowledged. Also, it is known that one infection can predispose to another, for example, measles as discussed above, influenza and bacterial pneumonias, and varicella and invasive group A streptococcal disease. Therefore, beneficial effects of vaccines beyond the target pathogens are also likely to occur by virtue of these mechanisms.

Although the WHO concluded that the currently available evidence is not sufficiently strong to drive changes in current global immunisation policy, the results of the systematic review by Higgins *et al* demonstrate the compelling need for new data to assess the importance of NSE from high-quality trials comparing the effects of different sequences of vaccinations on all-cause mortality. Generation of better evidence will be challenging both logistically and financially as a result of the difficulty of conducting sufficiently large and complex studies in the most resource poor settings.

Assuming global mortality in early childhood continues to fall as a result of economic development, vaccination, improved nutrition and provision of clean water, the potential impact of NSE of vaccines could diminish. However, some regions will doubtless continue to suffer considerable infectious mortality over the next few decades and all efforts to maximise the benefits of vaccination, including investigation of the potential of beneficial NSE, should continue.



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AF undertakes research studies and trials of vaccines funded by governments, charities and industry. He is a member of the UK Department of Health's Joint Committee on Vaccination, Chair of the WHO European Technical Advisory Group of Experts in which capacity he attends SAGE and President of the European Society for Paediatric Infectious Diseases, which receives sponsorship for its annual meeting from vaccine manufacturers.

NC is leading a large RCT of BCG in infants (ClinicalTrials.gov Identifier NCT01906853). He is a member of the WHO SAGE Working Group on BCG Vaccines. He is a Board member of the European Society for Paediatric Infectious Diseases, which receives sponsorship for its annual meeting from vaccine manufacturers.

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